

# Role of DNA Repair Inhibition in Lead- and Cadmium-induced Genotoxicity: A Review

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Compounds of lead and cadmium have been shown to be carcinogenic to humans and experimental animals. However, the underlying mechanisms are still not understood. In mammalian cells in culture, lead(II) is weakly mutagenic after long incubation times and generates DNA strand breaks only after treatment with high, toxic doses. Cadmium(II) induces DNA strand breaks and chromosomal aberrations, but its mutagenic potential is rather weak. However, both metals exert pronounced indirect genotoxic effects. Lead(II) is comutagenic towards UV and *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) and enhances the number of UV-induced sister chromatid exchanges in V79 Chinese hamster cells. With regard to DNA repair, lead(II) causes an accumulation of DNA strand breaks after UV-irradiation in HeLa cells, indicating an interference with the polymerization or ligation step in excision repair. Cadmium(II) enhances the mutagenicity of UV light in V79 Chinese hamster cells and an increased sensitivity toward UV light is observed in various rodent and human cell lines. Furthermore, an inhibition of unscheduled DNA synthesis after UV-irradiation and a partial inhibition of the removal of UV-induced DNA lesions has been shown. For both metals, the indirect genotoxic effects are observed at low, non-toxic concentrations, suggesting that an interference with DNA repair processes may be predominant at biologically relevant concentrations. This might also explain the conflicting results of epidemiological studies obtained for both metals. Possible mechanisms of repair inhibition are discussed. — Environ Health Perspect 102(Suppl 3):45–50 (1994).

Key words: lead, cadmium, genotoxicity, comutagenicity, DNA repair

## Introduction

Even though the toxic effects of lead and cadmium compounds have been studied for many years, inconsistent results have been published about their mutagenic, clastogenic, and carcinogenic properties.

Inorganic lead compounds are classified as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC) (1). This classification is based on results from animal studies, in which lead compounds caused mainly renal tumors in rats and mice after different routes of delivery (2). Additionally, lead acetate, lead subacetate, and lead oxide enhanced the number of tumors induced by some organic compounds, including 2-(ethylnitrosamine)ethanol (3) and *N*-(4'-fluoro-4-biphenyl)-acetamide (4). In contrast, the evidence for carcinogenicity to humans is still inadequate. While no excess of overall cancer deaths in workers in lead-related industries was observed in one study (5), an elevated number of deaths from all malignant neoplasms in smelter

workers but not in battery plant workers was reported (6); reevaluated by IARC, these data were found to be not significant (2). Much work has been done to determine the clastogenicity of lead compounds by investigating chromosomal aberrations in lymphocytes of lead-exposed workers. However, the results remain contradictory, which might be due in part to differences in culture conditions (7). Furthermore, it is not clear whether inorganic lead compounds exert clastogenic effects themselves, or whether they enhance chromosomal aberrations induced by compounds that occur simultaneously or arise during cell culturing (8–10).

Both soluble compounds of cadmium(II) such as CdCl<sub>2</sub> and CdSO<sub>4</sub>, as well as insoluble compounds like CdS and CdO are carcinogenic in experimental animals (11,12). In rats, the number of tumors induced by dimethylnitrosamine was enhanced by subsequent treatment with CdCl<sub>2</sub> (13). Several epidemiological studies point toward a higher frequency of tumors of exposed humans (14–16) and an increased incidence of renal cancer in smokers (17). A recent follow up of one of the studies (14) in a cadmium smelter in the United States confirmed a statistically significant excess in mortality from lung cancer (18,19). However, since the interpretation of epidemiological studies is always complicated by simultaneous exposures to other toxic or genotoxic agents (like arsenic in the cad-

mium smelter), studies in cultured cells are important to establish possible mechanisms of genotoxic action.

Regarding the genotoxicity in bacterial and mammalian cells in culture, the results are still conflicting: in bacteria, both cadmium(II) and lead(II) are not mutagenic, and in mammalian cells mutagenic effects are rather weak. In contrast, there are indications for rather indirect mechanisms of genotoxicity, which may be due to an interaction with DNA repair processes. The aim of the present article is to summarize both direct and indirect genotoxic effects of cadmium(II) and lead(II) and to elucidate the role of DNA repair inhibition in the genotoxicity of compounds of both metals.

## Direct Genotoxic Effects

### Lead(II)

The direct genotoxic potential of lead(II) in mammalian cells is weak and mainly restricted to toxic doses. In V79 Chinese hamster cells, both soluble and insoluble compounds of lead have been shown to be weakly mutagenic at the *hprt* locus after 5 days of incubation; additionally, the number of cell transformations in Syrian hamster embryo cells was enhanced after treatment with lead acetate (20). This weak mutagenic potential has been confirmed recently, where lead acetate was mutagenic at a toxic dose at the *E. coli gpt* locus transfected to V79 cells (21). In V79

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**Table 1.** Modulation of genotoxicity and interaction with DNA repair by lead(II) in mammalian cells.

Lead(II) in combination with	Cell line	Dose, $\mu$ M	Effect	Reference
UV light	V79	0.5–3	Enhanced mutation frequency ( <i>hprt</i> )	Hartwig et al. (22)
	V79	400	Enhanced mutation frequency ( <i>E. coli gpt</i> )	Roy and Rossman (27)
	V79	1–10	Enhancement of sister chromatid exchanges	Hartwig et al. (22)
	HeLa	500	Accumulation of DNA strand breaks during repair	Hartwig et al. (22)
MNNG	V79	400	Enhanced mutation frequency ( <i>E. coli gpt</i> )	Roy and Rossman (27)
X-rays	HeLa	250	Inhibition of recovery of DNA synthesis after X-irradiation	Skreb and Habazian-Novak (32)

MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

cells, neither lead acetate (20,22) nor lead sulfide (20) induced a significant number of sister chromatid exchanges. Furthermore, no DNA strand breaks or DNA-protein cross-links could be detected by alkaline elution (20) or nucleoid-sedimentation (22). In contrast, at toxic doses lead acetate and lead nitrate induced DNA breaks determined by nick translation (21).

### Cadmium(II)

In bacterial test systems, cadmium(II) was mostly not mutagenic (23). However, this may be due to a reduction of bioavailability of cadmium(II) by interaction with media components (24). In V79 Chinese hamster cells, CdCl<sub>2</sub> induced DNA single-strand breaks, DNA-protein cross-links (25), and chromosomal aberrations (26). Furthermore, it enhanced the number of mutations at the thymidine kinase (*tk*) locus in mouse lymphoma L51784/TK<sup>+</sup> cells (27) and at the *hprt* locus in V79 cells (25,28). Concerning the generation of DNA single-strand breaks and chromosomal aberrations, the involvement of reactive oxygen species has been shown (29,30), which might be mediated by a decrease in intracellular glutathione induced by cadmium(II) at similar concentrations (31).

### Indirect Genotoxic Effects and Inhibition of DNA Repair

#### Lead(II)

Although inorganic lead compounds exhibit only a weak mutagenic potential,

they show more pronounced comutagenic activities in combination with other DNA-damaging agents (Table 1). In V79 Chinese hamster cells, lead acetate enhanced the frequencies of UV-induced mutations at the *hprt* locus as well as sister chromatid exchanges (22). The comutagenicity toward UV light was confirmed recently in G12 cells; additionally, an enhancement of mutations induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) by lead(II) was shown (21). These effects seem to be due to an interference with DNA repair processes, since lead ions caused an accumulation of DNA strand breaks after UV irradiation, as shown in human HeLa cells (22). Since the repair of UV-induced DNA damage is mediated by the nucleotide excision repair pathway, which is characterized by the transient occurrence of DNA strand breaks as a result of incisions at the sites of damage, an accumulation of breaks points toward an inhibition of the polymerization or ligation step by lead(II). Regarding the effects of lead ions on the repair of X-ray induced-DNA damage, the results are controversial. While 250  $\mu$ M lead chloride inhibited the recovery of DNA synthesis after X-irradiation in HeLa cells, indicating an inhibition of repair of X-ray-induced DNA lesions (32), no effect of lead acetate on the resealing of X-ray-induced DNA strand breaks was observed in the same cell line (33). However, since lead ions are taken up only slowly in mammalian cells (22), the preincubation time of 30 min

might not have been sufficient in the latter study. The inhibition of DNA repair as one mechanism of genotoxicity has also been supported by several articles concerning the interaction of lead ions with isolated enzymes involved in DNA processing and repair. In this context, a decreased fidelity of the DNA polymerase from avian myeloblastosis virus (AMV) in the presence of lead chloride has been shown (34); however, the concentration of 4 mM applied was very high. The results concerning a possible inhibition of DNA polymerases are controversial. Polymerase  $\alpha$  and RNA polymerase II isolated from HeLa cells were inhibited by 80 and 150 mM lead acetate. Similarly, DNA- and RNA-synthesis was reduced in intact nuclei from HeLa cells after incubation with similar concentrations of the metal compound. No inhibition of DNA- or RNA-synthesis, however, was found in intact HeLa cells after exposure to 500  $\mu$ M lead acetate for up to 18 hr, indicating a lack of bioavailability of lead ions (35). While an inhibition of isolated human polymerase  $\beta$  at concentrations as low as 10 mM lead nitrate was demonstrated (36), an increase in DNA polymerase  $\beta$  mRNA as observed in proliferating hepatocytes after lead nitrate administration to male Wistar rats (37).

### Cadmium(II)

In addition to the direct genotoxic effects described above, one other line of evidence suggests the enhancement of genotoxicity of other DNA damaging agents by cadmium(II), possibly by interfering with DNA repair processes involved in the removal of DNA damage induced by alkylating agents or UVC irradiation. These effects are summarized in Table 2.

In bacterial test systems, a comutagenic effect of cadmium(II) in combination with methyl nitrosourea (MNU) was observed in *S. typhimurium* (38,39) and confirmed in *E. coli* (40). The latter effect was due to the inactivation of the *O*<sup>6</sup>-methylguanine-DNA methyl transferase (MGase) (41). This protein protects cells from cytotoxic and mutagenic DNA damage after exposure to alkylating agents by accepting the methyl group from *O*<sup>6</sup>-methylguanine and thereby reversing the damage. Furthermore, the methylated protein induces its own biosynthesis by transcriptional activation of the *ada* gene (42). The effect of cadmium(II) on this protein was attributed to the inhibition of its transcriptional activity, possibly by binding to critical methyl group acceptor sites (41). When the MGase was isolated from *E. coli*, rat

**Table 2.** Modulation of genotoxicity and interaction with DNA repair by cadmium(II).

Cadmium (II) in combination with	Cell line	Dose $\mu$ M	Effect	Reference
Bacterial test systems				
MNU	<i>E. coli</i>	10–500	Enhanced mutation frequency	Takahashi et al. (40)
MNNG	<i>S. typhimurium</i>	250–500	Enhanced mutation frequency	Mandel and Ryser (38)
MMS	<i>E. coli</i>	250–500	Enhanced mutation frequency	Takahashi et al. (56)
Mammalian cells				
UV light	V79	0.5–2	Enhanced mutation frequency ( <i>hprt</i> )	Hartwig and Beyersmann (28)
	Human fibroblasts	5	Reduction of colony forming ability	Nocentini (45)
	Human fibroblasts	4	Inhibition of unscheduled DNA synthesis	Nocentini (45)
	Human fibroblasts	4	Accumulation of DNA strand breaks during repair	Nocentini (45)
	HeLa	5	Inhibition of thymine-thymine dimer removal	Snyder et al. (33)
benzo(a)pyrene	SHE	1.9	Enhancement of morphological transformations	Rivedal and Sanner (57)

Abbreviations: MNU, methyl nitrosourea; MNNG, *N*-methyl-*N'*-nitro-*N*-nitroguanidine; MMS, methyl methanesulfonate.

**Table 3.** Cellular damage induced by lead(II) in V79 cells.

Observed effect	Dose, $\mu$ M	Treatment time hr	CFA % of control	Reference
Comutagenicity with UV, <i>hprt</i>	0.5–3	48	not reduced	Hartwig et al. (22)
( <i>E. coli gpt</i> <sup>a</sup> ) with MNNG	400	24	not reduced	Roy and Rossman (21)
( <i>E. coli gpt</i> <sup>a</sup> )	400	24	not reduced	Roy and Rossman (21)
Mutations <i>hprt</i> ( <i>E. coli gpt</i> <sup>a</sup> )	500–2000	120	94–56	Zelikoff et al. (20)
	1700	120	40–50	Roy and Rossman (21)
DNA strand breaks (nick translation <sup>a</sup> )	1700	120	40–50	Roy and Rossman (21)

<sup>a</sup> G12 cells (V79 cells carrying an *E. coli gpt* gene).

liver, or human cells, it was inactivated by cadmium(II) (43,44). However, no data concerning the interaction of cadmium with alkylating agents in mammalian cells are currently available.

In contrast, there are some indications for an interference by cadmium(II) with UV-induced DNA damage in mammalian cells. We observed an enhancement of UV-induced mutagenicity by CdCl<sub>2</sub> in V79 cells (28) as well as an increase in UV-induced cytotoxicity by nontoxic concentrations of CdCl<sub>2</sub> in various cell lines including V79, CHO, HeLa, and human fibroblasts. The enhancement of cytotoxicity could be attributed to an interference with DNA repair processes, since no altered sensitivity toward UV irradiation in the presence of cadmium(II) was detected in repair-deficient human fibroblast Xeroderma pigmentosum complementation group A (XPA) (Hartwig and Beyersmann, unpublished). Cadmium(II) caused a reduction in unscheduled DNA synthesis (UDS) and an accumulation of DNA strand breaks following UV irradiation in human fibroblasts (45). Furthermore, a partial inhibition of the removal of UV-induced pyrimidine dimers by cadmium(II) was shown, while the resealing of DNA strand breaks generated by X-rays was not affected (33). Concerning isolated enzymes involved in DNA replication and repair, an inhibition of DNA polymerase  $\beta$  at low concentrations of cadmium acetate was observed (36), as well as a decrease in the fidelity of DNA polymerization in the presence of cadmium(II) (34).

### Relevance of Genotoxicity Enhancing Effects and Repair Inhibition in Mammalian Cells

The results described so far demonstrate that cadmium(II) and lead(II) are genotoxic themselves and enhance the genotoxicity of other DNA damaging agents. However, to assess the relevance of the observed effects, the concentrations applied have to be considered. A comparison among the different types of cellular damage, the effective concentrations, and the reduction in colony-forming ability as a parameter of cytotoxicity after the respective treatments is presented in Tables 3 and 4. Since different cell lines vary considerably in their sensitivities toward metal compounds, only those effects published for V79 Chinese hamster cells have been listed.

Lead(II) enhanced the frequencies of UV-induced mutations and sister chromatid exchanges at very low, nontoxic con-

**Table 4.** Cellular damage induced by cadmium(II) in V79 cells.

Observed effect	Dose [mM]	Treatment time (hr)	CFA (% of control)	Reference
Comutagenicity with UV (hprt)	0.5–2	19	Not reduced	Hartwig & Beyersmann (28)
Mutagenicity (hprt)	1–3	24	100–70	Ochi & Osawa (25)
	2	19	Not reduced	Hartwig & Beyersmann (28)
Chromosomal aberrations	10–50	2	50–0	Ochi et al (26)
DNA strand breaks (alkaline elution)	20–200	2	20–0	Ochi & Osawa (25)
Reduction of GSH	20–200	2	20–0	Ochi et al (37)

centrations (22). Even though the doses applied in the second study were much higher, the authors confirmed a comutagenic activity of lead(II) in combination with UV light and MNNG on conditions in which the colony-forming ability was not reduced (21). In neither study was lead(II) alone mutagenic on these conditions; mutations as well as DNA strand breaks occurred only after long-term treatment at much higher cytotoxic doses (20,21).

Regarding cadmium(II), comutagenicity in combination with UV light was observed at low doses, which did not lead to a reduction in colony-forming ability. The mutagenicity of cadmium itself occurred at similar low concentrations; however, the mutagenic potential was rather weak compared to the comutagenic effects. In contrast, chromosomal aberrations and DNA single-strand breaks were generated only at considerably higher concentrations. Even though the treatment time of 2 hr was comparatively short, these effects arose at highly toxic concentrations only. Even under these conditions, a reduction in cellular glutathione content was generated by cadmium(II), which might render the cells more susceptible to damage by oxygen free radicals.

### Possible Mechanisms of Repair Inhibition

There are several possible mechanisms by which cadmium(II) and/or lead(II) might interfere with DNA repair processes. While the comutagenicity of cadmium(II) in combination with alkylating agents in bacteria

could be attributed to the inactivation of the *O*-methylguanine-DNA methyl transferase, the inhibition of excision repair by both metals is not yet understood.

For lead and cadmium ions, the accumulation of DNA strand breaks after UV irradiation obtained from nucleoid sedimentation in HeLa cells [(22); Hartwig and Beyersmann, unpublished] and in the case of cadmium from alkaline elution in human fibroblasts (45), suggest an inhibition of the polymerization or ligation step in excision repair. This could be due to either enzyme inactivation or changes in DNA structures, preventing repair enzymes from binding. The interactions of lead and cadmium ions were investigated with isolated DNA polymerase  $\beta$  (36). The authors found that for both metals the inactivation was mediated by the reaction with the enzyme itself, not with the template primer or the deoxynucleoside triphosphate substrate. One reason for this inhibition could be the competition with zinc ions, which are essential in DNA polymerases (46). Furthermore, other DNA binding proteins involved in excision repair have been shown to contain zinc-finger motifs in their DNA binding domain (47). For example, the zinc-finger structure in the recently cloned and characterized Xeroderma pigmentosum group A complementing (XPAC) protein has been shown to be essential for the excision repair process (48). In support of a possible competition between cadmium(II) and zinc(II) in DNA repair, a partial reversibility of repair inhibition by cadmium(II) (unscheduled DNA synthesis, accumulation of DNA strand breaks) in human fibroblasts was observed, when zinc(II) was given simultaneously in a 10-fold

molar excess. Additionally, the enhancement of UV-induced cytotoxicity by cadmium(II) was reversed by zinc(II) (45) an effect which also has been observed by us [Hartwig and Beyersmann, unpublished]. However, the interpretation of these results is complicated by the fact that cadmium(II) and zinc(II) compete at the level of cellular uptake (49) and the effect might therefore be due to a diminished uptake of cadmium in the presence of zinc(II). With isolated polymerase  $\beta$ , the inhibition by lead(II) or cadmium(II) was not reversible by the subsequent addition of a 10-fold molar excess of zinc(II), and the inhibitory effect of cadmium(II) was even smaller when the polymerase was activated by zinc(II) instead of manganese(II) (36). Whether or not an inhibition of the polymerization step is the underlying mechanism of repair inhibition by lead(II) or cadmium(II) in intact cells has yet to be elucidated. Even though polymerase  $\beta$  is involved in cellular repair synthesis, it is thought to act mainly on short gaps generated during base excision repair (50), whereas after UV irradiation DNA polymerase  $\delta$  catalyzes the formation of repair patches (51,52). Besides a direct interaction with repair enzymes, lead and cadmium ions might also interfere with calcium-regulated processes involved in the regulation of DNA replication and repair. In this context, the specific calmodulin-inhibitor trifluoperazine has been shown to inhibit the repair of UV-induced pyrimidine dimers (53) and to potentiate the lethality of bleomycin (54). Even though manifold interactions between lead and calcium concerning uptake, calcium homeostasis and substitution of lead(II) for calcium(II) have been investigated (55), their potential role in repair inhibition has yet to be elucidated.

### Conclusions

In summary, the results suggest that for compounds of both metals, the indirect genotoxic effects may be predominant at biologically relevant doses. Especially for lead(II), the interference with DNA repair processes seems to be the main mechanism of genotoxicity. This might help to explain the conflicting results of epidemiological studies with regard to the clastogenic and carcinogenic potential of lead compounds. In mammalian cells, both cadmium(II) and lead(II) have been shown to interfere with the repair of UV-induced DNA damage. Since the removal of these DNA lesions is mediated by the nucleotide excision repair system, which is generally acting on bulky DNA damage, it may be expected that lead and cadmium also increase the genotoxicity when combined with other DNA damaging agents.

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